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| (54) Title: METHOD AND APPARATUS FOR ANALYZING NUCLEIC ACIDS (57) Abstract A method and apparatus for analyzing nucleic acids includes immobilizing nucleic probes at specific sites within a microchannel structure and moving target nucleic acids into proximity to the probes in order to allow hybridization and fluorescence detection of specific target sequences. | | |

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METHOD AND APPARATUS FOR ANALYZING NUCLEIC ACIDS

This invention was made with government support under Contract No. DE-AC05-840R21400 awarded by the U.S. Department of Energy to Lockheed Martin Energy Systems, Inc. and the government has certain rights in
5 this invention.

BACKGROUND OF THE INVENTION

Field of The Invention

The present invention relates generally to medical and/or biological testing and devices for
10 performing same, and more particularly, to a method and apparatus for analyzing minute amounts of nucleic acids for the presence of specific nucleotide sequences. Single-strand DNA probes are bound to specific regions of microchannels in a glass microchip device. Sub-
15 microliter volumes of nucleic acid solutions, buffers and other reagents are transported through the channels under electrokinetic or hydraulic control. Hybridization of target nucleic acid sequences to complementary probes is detected using either
20 fluorescent labels or intercalating fluorescent dyes.

DESCRIPTION OF THE RELATED ART

Hybridization analysis is typically performed in microtiter plate wells or on planar surfaces that contain arrays of DNA probes. Chemical manipulations
25 are required to bring about a hybridization test and to

detect the results. These manipulations presently include washing or dipping planar arrays into the appropriate chemicals.

5 The aforementioned procedures suffer from many drawbacks. For example, they are wasteful of expensive reagents and limited sample volumes. Moreover, they are generally not compatible with efficient automation strategies and thus tend to be time consuming.

10 A continuing need exists for methods and apparatuses that limit the use of expensive reagents and priceless samples, while simplifying the overall procedures to require smaller samples and fewer processing steps.

SUMMARY OF THE INVENTION

15 An object of the present invention is to provide a method and apparatus for analyzing nucleic acids which simplifies chemical manipulations required to bring about a hybridization test when performing DNA diagnostics in biomedical, forensic, and research
20 applications.

Another object of the present invention is to provide a method and apparatus for analyzing nucleic acids which minimizes the use of expensive reagents and limited sample volumes.

25 Another object of the present invention is to provide a method and apparatus for analyzing nucleic acids which avoids the necessity of pre-labeling a target DNA and increases the sensitivity of hybrid detection by reducing background fluorescence due to
30 non-specific surface adsorption of labeled target DNA.

Still another object of the present invention is to provide a method and apparatus for analyzing nucleic acids which significantly extend the usefulness of hybridization diagnostics by allowing its application to much smaller samples and facilitating automated processing.

These and other objects are met by providing an apparatus for analyzing nucleic acids which includes a microchip having a microchannel structure formed therein, at least one portion of the microchannel structure having at least one site capable of affixing thereto a probe, and a plurality of reservoirs in communication with the microchannel structure for introducing at least one of, or a mixture of, a reagent, analyte solution, and buffer.

In another aspect of the invention, a method of analyzing nucleic acids includes bonding oligonucleotide probes to a microchannel formed in a microchip, adding target nucleic acids and fluorescent stains to the microchannel, and detecting hybridization by fluorescence staining of double-stranded DNA.

These together with other objects and advantages which will be subsequently apparent, reside in the details of construction and operation as more fully hereinafter described and claimed, with reference being had to the accompanying drawings forming a part hereof, wherein like numerals refer to like elements throughout.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic view of an apparatus for analyzing nucleic acids according to a preferred embodiment of the present invention;

5 Figures 2 and 3 are schematic views of different arrangements of nucleic acid hybridization probes in microchannels;

10 Figure 4 is a schematic view of a microchip and microchannel structure according to another preferred embodiment of the present invention;

Figure 5 is a schematic view of a microchip of the present invention;

15 Figure 6 is a photomicrograph showing discrimination of target and non-target DNA at the intersection of microchannels in the inset area of Figure 5 after dsDNA staining with fluorescent dye;

Figure 7 is a schematic view of another apparatus for analyzing nucleic acids according to a preferred embodiment of the present invention; and

20 Figure 8 shows fluorescence image profiles of two probe channels after ds-DNA staining with fluorescent dye.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

25 Referring to Figure 1, a microchip 16 includes a glass substrate 18 and a cover plate 20 which covers a microchannel structure 22 formed in the upper surface of the substrate 16. The cover plate 20 is permanently bonded to the substrate 18. Both the substrate 18 and cover plate 20 are preferably made of clear glass, and

30 the substrate may preferably be made from a standard

microscope slide. Alternative construction materials could include plastics (such as polypropylene, polycarbonate, or polymethylmethacrylate), silicon, or sapphire.

5 The microchannel structure 22 is formed using standard photolithographic techniques, and includes a longitudinal microchannel manifold portion 24, a first transverse microchannel portion 26 forming an intersection 28 with the longitudinal portion 24, and a
10 second transverse microchannel portion 30 forming an intersection 32 with the longitudinal portion 24.

 First and second reservoirs 34 and 36 are in fluid communication with opposite ends of the longitudinal portion 24. The opposite ends act as ports to
15 introduce the contents of the reservoirs 34 and 36 into the microchannel structure 22. Each reservoir can be a cylindrical container open at its opposite axial ends, with the ends of the longitudinal portion 24 being in fluid communication with the bottom of the container.

20 Third and fourth reservoirs 38 and 40 are in fluid communication with opposite ends of the first transverse portion 26. The opposite ends act as ports to introduce the contents of the reservoirs 38 and 40 into the microchannel structure 22. Each reservoir 38
25 and 40 is similar in construction to the other reservoirs, with the ends of the first transverse portion being in fluid communication with the bottom of each respective reservoir 38 and 40.

 Fifth and sixth reservoirs 42 and 44 are in fluid
30 communication with opposite ends of the second transverse portion 30. The opposite ends act as ports

to introduce the contents of the reservoirs 42 and 44 into the microchannel structure 22. Each reservoir 42 and 44 is similar in construction to the other reservoirs, with the ends of the second transverse portion being in fluid communication with the bottom of each respective reservoir 42 and 44.

One or more types of single-stranded DNA probes 46 are attached at individual sites within the microchannel portion 24 of the microchannel structure 22. The design and fabrication of microchips and the electrokinetic transport of fluids through the microchannels is described in U.S. Serial No. 08/283,769, filed August 1, 1994, hereby incorporated by reference. The microchips described therein include planar, glass substrates into which the microchannels are etched photolithographically. The reservoirs typically hold analyte solutions, buffers, reagents, etc. Typical microchannel dimensions are 10 μm by 50 μm (depth x width), although channel widths of 1 μm to > 100 μm and channel depths of < 1 μm to > 100 μm may be used. Voltages are applied to solutions as described in the aforementioned application to produce electroosmotic flow of fluids or electrophoretic migration of charged species through the channels. Alternatively, pressure (or vacuum) may be applied to one or more fluid reservoirs to cause reagent flow through the channels.

The individual DNA probes may be arranged in a linear pattern, as shown in Figure 2. An alternative embodiment is shown in Figure 3, wherein the 46' are arranged in a two-dimensional array in a widened area

48 of the channel portion 24'. Fluid flow is in the direction indicated by arrows.

Typically, oligonucleotide probes ten to thirty nucleotides long are used for hybridization analysis, although much longer probes, such as DNA restriction fragments or cDNA sequences of > 100 nucleotide length, may be used in certain applications.

Oligonucleotide probes may be immobilized by covalent chemical linkage to the surface. In general, such linkage involves derivatization of the glass surface with a silane coupling agent, such as 3-aminopropyltriethoxysilane or 3-glycidoxypropyltrimethoxysilane. An oligonucleotide probe bearing an alkylamine group at the 5' or 3' end may then be linked to the surface by direct reaction of its terminal amine with a silane epoxy group or by cross linking the silane and oligonucleotide amines using glutaraldehyde or other amine-reactive bi-functional compounds.

Other immobilization method may also be used. For example, surface-immobilized avidin or streptavidin may be used to bind biotinylated probes. Non-covalently adsorbed oligonucleotides on glass surfaces have also been shown to hybridize to target sequences.

In the preferred fabrication method, the probes are attached to the open microchip channels and the cover plate is then bound to the substrate by a low temperature technique which does not damage the biomolecules. Such a low temperature bonding technique is described in copending application serial no. 08/645,497, entitled " Low Temperature Material Bonding

Technique" by J.M. Ramsey, R.S. Foote, and H. Wang, which is incorporated herein by reference. Individual probes may be applied to specific sites in the channels by micro-pipeting or other means, such as ink-jet printing. The separation of individual probes may be facilitated by preparing the surface with a pattern of reactive, hydrophilic sites separated by non-reactive, hydrophobic areas. For example, the glass surface may be treated with an alkyltrialkoxysilane to produce a non-reactive, hydrophobic surface. Photolithography and chemical etching or laser ablation may be used to remove the silane layer and expose the glass substrate in a pattern of separated spots. These spots may then be treated with a silane coupling agent as described above to produce reactive, hydrophilic spots. An aqueous probe solution applied to an individual spot would be confined to its hydrophilic site and thus prevented from mixing with different probe solutions in adjacent spots. The intervening hydrophobic regions would also prevent probe mixing in the case of the other immobilization methods described above.

Alternatively, the probes may be attached to specific sites in the channels after standard high-temperature cover plate bonding. Three methods of achieving this are provided as examples:

(1) The functional group of the silane linker (e.g., the amino function of 3-aminopropylsilane) may be blocked with a photolabile protective group. The silane linkers are then de-protected at specific positions in the channel by exposure to light through the cover plate using a photolithographic mask or

focused beam. Cross linkers and probes passed through the channel would react only at de-protected sites. A series of separate de-protection and addition steps are used to attach a number of different probes to
5 individual sites.

(2) An array of oligonucleotide probes may be photochemically synthesized in situ in a parallel fashion.

(3) A channel manifold may be designed to allow
10 the addition of an individual probe to a given branch or segment of the manifold by controlling fluid flows.

In the preferred methodology, nucleic acids, buffers and dyes are electrokinetically driven through the microchannels containing the immobilized probes.
15 For example, the following sequence of operations can be used with the device schematically illustrated in Figure 4. As seen in Figure 4, a microchip 50 includes a microchannel structure 52 connected to a nucleic acid sample reservoir 54, a buffer reservoir 56, a dye
20 reservoir 58, dye buffer reservoir 60, and waste reservoir 62. A hybridization chamber 64 is disposed in the microchannel structure 52 between first and second transverse portions 66, 68 of the microchannel structure.

25 A voltage is applied between reservoir 54 which contains the nucleic acid sample being analyzed and reservoir 56 containing nucleic acid buffer. For buffers containing a high NaCl concentration (desirable for rapid nucleic hybridization) the polarity of
30 reservoir 56 is positive relative to reservoir 54 and the negatively charged nucleic acids

electrophoretically migrate from reservoir 54 to reservoir 56, passing through the hybridization chamber 64. Alternatively, a nucleic acid solution containing a low salt concentration may be electroosmotically transported into the hybridization chamber by applying a positive voltage at reservoir 54 relative to reservoir 56. Because electroosmotic flow toward reservoir 56 is high relative to electrophoretic migration toward the positive electrode, the net movement of nucleic acids will be toward reservoir 56 in the later case. The use of electroosmotic flow versus electrophoretic migration will depend on a number of factors, and may vary depending on the type of sample being analyzed. The term "electrokinetic transport" includes both electroosmotic flow and electrophoretic migration.

After the DNA sample reaches equilibrium over the probe sites, the voltage may be discontinued while hybridization occurs. A double-strand-DNA-specific (dsDNA-specific) fluorescent dye is then electrokinetically transported through the hybridization chamber 64 by applying voltages to fluid reservoir 58 which contains a dye and reservoir 60 containing a dye buffer. Because high salt concentrations are not normally required or desirable for this step, electroosmotic flow is the preferred method of dye addition and the polarity of reservoir 58 will normally be positive relative to reservoir 60. Several fluorescent double-strand-specific nucleic acid stains are commercially available. Many of these stains are positively charged so that their

electrophoretic migration will be in the same direction as the electroosmotic flow.

Alternatively, the nucleic acids being analyzed may be pre-labeled with fluorescent groups by well known procedures. Although this later method can lead to higher background fluorescence, it may be preferred in cases where probes contain self-complementary sequences that can result in stable duplex formation and dye binding by the probe itself.

Variations in the chip design and analysis procedure are possible. For example, electrokinetically driven washing steps may be included before and/or after the dye addition step by applying appropriate voltages between the buffer reservoirs and a waste reservoir 62. Nucleic acid and dye solutions might also be added simultaneously to the hybridization chamber. As an alternative to electrokinetically driven fluid manipulation, hydraulic pressure or vacuum may be applied to appropriate reservoirs to control the flow of solutions through the microchannels.

After completion of the hybridization and dsDNA staining steps, if used, the hybridization chamber is examined for the presence of fluorescently labeled sites by illumination with exciting light through the cover plate. An epifluorescence microscope and CCD camera may be used, as described below, to obtain a fluorescence image of the entire chamber or portion thereof. Scanning confocal fluorescence microscopy may also be used.

The following examples incorporate the apparatus and methodology of the present invention. Each

involves the steps of (1) covalently bonding oligonucleotide probes to microchannels, (2) adding target nucleic acids and fluorescent stains to microchannels by electrokinetic flow, (3) detecting hybridization by fluorescence staining of double-stranded DNA, and (4) discriminating target and non-target nucleic acids.

Example 1

10 A 16-mer oligodeoxynucleotide probe sequence containing a 5'-(6-aminohexyl)phosphate [H₂N-CH₂)₆-5'-pCGGCACCGAGTTTAGC-3'] was covalently attached to the hybridization chamber of a prototype microchip similar to that shown in Figure 4 by
15 glutaraldehyde cross linking with the 3-aminopropylsilane-derivatized glass surface. A complementary 16-mer (target sequence) oligodeoxynucleotide in 6 x SSC buffer was then electrophoretically added to the hybridization chamber
20 by applying 0.5 kV between reservoir 56 and reservoir 54 (positive electrode at reservoir 54) for thirty minutes. A dsDNA-specific fluorescent dye (TOTO-1, Molecular Probes) in 10 mM Tris-borate buffer, pH 9.2, was then electroosmotically added to the chamber by
25 applying 1.0 kV between reservoir 60 and reservoir 58 for 30 minutes. The chip was examined by video microscopy using laser excitation (514 nm) of fluorescence. Bright fluorescence due to the dsDNA-bound dye was observed in the hybridization chamber
30 relative to channels not exposed to the target DNA. The image was recorded on video tape.

In a subsequent similar experiment using the ds-DNA specific dye, PicoGreen (Molecular Probes), quantification by CCD imaging and analysis showed a 10-fold increase in fluorescence intensity when staining was carried out after hybridization of the target DNA, relative to the intensity observed by staining prior to the hybridization step.

Example 2

The 16-mer oligonucleotide probe of Example 1 was uniformly bound to the channels of a cross-channel chip shown schematically in Figure 5 by glutaraldehyde cross-linking. Solutions (50 μ M) of the complementary (target sequence) 16-mer oligodeoxynucleotide (T) and a non-complementary (non-target sequence) 16-mer oligodeoxynucleotide (N) in phosphate-buffered saline (PBS) were then added to separate channels as indicated in Figure 5, by applying suction at W for 10 minutes. The channels were then washed with buffer and dsDNA-specific dye solution (PicoGreen, Molecular Probes) was added to all channels for five minutes. The cross-channel intersection was examined by epifluorescence microscopy using a mercury lamp illumination source and FITC filters. A 1.0 second CCD exposure, shown in Figure 6 as the insert of the broken line area of Figure 5, showed intense fluorescence (dark regions) in the channel exposed to target DNA relative to that of channels exposed to non-target DNA or buffer.

In a similar experiment using laser induced fluorescence imaging, as described in co-pending application serial no.08/800,241, entitled "Method and

Apparatus for Staining Immobilized Nucleic Acids" by J.M. Ramsey and R.S. Foote, incorporated herein by reference, signal intensity from channels exposed to target DNA was 10-fold greater than from channels exposed to non-target DNA or buffer.

Example 3

Two 16-mer probes [H₂N-(CH₂)₆-5'-GCTAAACTCGGTGCCG-3' (Probe 1)] and [H₂N-(CH₂)₆-5'-pCGGCACCGAGTTTAGC-3' (Probe 2)] were immobilized in separate channels of a cross-channel chip as indicated in Figure 7. In Figure 7, the "T" reservoir is for target DNA, "B" is for PBS buffer and "W" is for waste.

A solution of 16-mer oligonucleotide (50 nM oligonucleotide in PBS) complementary to Probe 1 was induced to flow through both channels for a total of 15 minutes by applying a vacuum at W. The channels were then washed with buffer and treated with a ds-DNA specific dye solution (PicoGree, Molecular Probes) for two minutes. After washing with 10 mM Tris-HCL (pH 8), one mM EDTA (TE) buffer for one minute, the channels were examined for laser-induced fluorescence using an argon ion laser at 488 nm and 100 milliwatts power. Quantitation by CCD imaging, shown in Figure 8, shows a 4 to 5-fold greater fluorescence in the Probe 1 channel than in the Probe 2 channel after subtraction of the background signal.

According to the above methods and apparatuses, hybridization analysis can be performed in a microchip structure that requires low instrumentation space and

extremely low sample/reagent volumes. The electrokinetic transport of samples and reagents facilitates automation of sample/reagent manipulations. Moreover, the detection of hybridization using double-
5 strand DNA-specific fluorescent dyes eliminates the target DNA labeling step associated with prior art techniques and increases detection sensitivity.

While the examples referred to above describe nucleic acid probes, the methodology and apparatuses
10 could also be used for other uses including, but not limited to, immobilized antibodies for micro-immunoassays. Numerous biomedical applications can be envisioned.

While the various embodiments have referred to
15 specific reservoirs containing specific reagents, buffers or samples, mixtures of two or more substances can be contained in individual reservoirs. For example, a reservoir can contain a mixture of reagent and buffer, buffer and sample, etc.

20 The many features and advantages of the invention are apparent from the detailed specification, and thus, it is intended by the appended claims to cover all such features and advantages of the invention which fall within the true spirit and scope of the invention.
25 Further, since numerous modifications and variations will readily occur to those skilled in the art, it is not desired to limit the invention to the exact construction and operation illustrated and described and accordingly, all suitable modifications and
30 equivalents may be resorted to, falling within the scope of the invention.

What is claimed is:

1. A method for analyzing nucleic acids comprising the steps of:
 - forming a microchannel structure in a substrate;
 - treating at least a portion of the microchannel
 - 5 structure to produce a pattern of reactive, hydrophilic sites;
 - applying a different aqueous probe solution to each hydrophilic site to couple a plurality of probes to the microchannel structure; and
 - 10 affixing a cover plate to the substrate thus enclosing the probes.
2. A method according to claim 1, further comprising electrokinetically transporting a target nucleic acid sample through the microchannel structure to the plurality of probes.
3. A method according to claim 1, further comprising hydraulically transporting a target nucleic acid sample through the microchannel structure to the plurality of probes.
4. A method according to claim 1, further comprising transporting a target nucleic acid sample to at least one of the probes, thus forming at least one hybridized site, delivering an intercalating dye to the hybridized
- 5 site, and probing the hybridized site for fluorescence emission.

5. A method according to claim 1, further comprising transporting a fluorescently-labeled target nucleic acid sample to at least one of the probes, thus forming at least one hybridized site, and probing the
5 hybridized site for fluorescence emission.
6. A method according to claim 4, wherein the delivering step comprises electrokinetically delivering the intercalating dye.
7. A method according to claim 4, wherein the delivering step comprises hydraulically delivering the intercalating dye.
8. A method according to claim 1, wherein the treating step includes treating a surface of the microchannel structure with a hydrophobic substance, thus forming a hydrophobic layer and a non-reactive,
5 hydrophobic surface, removing the hydrophobic layer in a way as to form a pattern of exposed sites, treating the exposed sites with a silane coupling agent to produce reactive, hydrophilic sites exposing oligonucleotide probes to the hydrophilic sites,
10 thereby binding the probes to the hydrophilic sites.
9. A method according to claim 8, wherein the hydrophobic substance is an alkyltrialkoxysilane.

10. A method according to claim 8, wherein the silane coupling agent is selected from the group consisting of 3-aminopropyltriethoxysilane and 3-glycidoxypropyltrimethoxysilane.
11. A method according to claim 8, wherein the oligonucleotide probe is modified with a linking group.
12. A method according to claim 11, wherein the linking group is an aminoalkyl group having the structure: $-(CH_2)_nNH_2$, with the alkyl chain length, n , having a value between 3 and 12, inclusive.
13. A method according to claim 12, wherein the aminoalkyl linking group is attached to the 5' terminus of the oligonucleotide.
14. A method according to claim 12, wherein the aminoalkyl linking group is attached to the 3' terminus of the oligonucleotide.
15. A method according to claim 8, wherein the step of binding the probe consists of treating 3-glycidoxypropylsilanated sites with an oligonucleotide probe modified with an aminoalkyl group, whereby the aminoalkyl group reacts with the silane epoxide group to bind the said oligonucleotide probe to the silanated site.

16. A method according to claim 8, wherein the step of binding the probe consists of treating 3-aminopropylsilanated sites with an amine-reactive crosslinker followed by treatment with an
5 oligonucleotide probe modified with an aminoalkyl group, whereby the said oligonucleotide probe is bound to the silanated site.

17. A method according to claim 8, wherein the removing step includes photolithographically removing the silane layer.

18. A method according to claim 1, further comprising electrokinetically moving target nucleic acids into the channel structure and detecting hybridization between the probes and the target nucleic acids.

19. A method according to claim 17, wherein the photolithographically removing step includes spin-coating an organic resist over the silane layer, patterning the resist so as to expose the silane layer
5 at selected sites, and removing the silane layer at the selected sites by chemical etching.

20. A method according to claim 17, wherein the photolithographic step includes removing the silane layer at selected sites by laser ablation.

21. A method for analyzing nucleic acids comprising the steps of:

forming a microchannel structure in a substrate;
affixing a cover plate to the substrate thus
5 enclosing the microchannel structure;
treating at least a portion of the microchannel structure to produce a pattern of reactive sites; and
applying a different aqueous probe solution to
each reactive site to couple a plurality of probes to
10 the microchannel structure.

22. A method according to claim 21, wherein the treating step includes treating at least a portion of the microchannel structure with a silane linker containing a photolabile protective group, exposing the
5 photolabile groups at specific positions in the microchannel to light through the cover plate, followed by covalently binding an oligonucleotide probe to said exposed positions.

23. A method according to claim 22, wherein the silane linker is an amine functional silane and the photolabile protective group is an amine protective group.

24. A method according to claim 22, wherein the oligonucleotide probe is modified with an aminoalkyl linking group.

25. A method according to claim 22, wherein covalently binding an oligonucleotide probe includes the steps of treating exposed aminosilane positions with an amine reactive crosslinker, followed by treatment with an aminoalkyl-modified oligonucleotide probe.

26. A method according to claim 21, further comprising electrokinetically transporting a target nucleic acid sample through the microchannel structure to the plurality of probes.

27. A method according to claim 21, further comprising hydraulically transporting a target nucleic acid sample through the microchannel structure to the plurality of probes.

28. A method according to claim 21, wherein the treating step includes controlling fluid flow through the microchannel structure to place individual probes in a given portion of the channel structure.

29. A method according to claim 21, further comprising transporting a target nucleic acid sample to at least one of the probes, thus forming at least one hybridized site, delivering an intercalating dye to the hybridized site, and probing the hybridized site for fluorescence emission.

30. A method according to claim 21, wherein the applying step includes transporting a fluorescently-labeled target nucleic acid sample to at least one of the probes, thus forming at least one hybridized site, and probing the hybridized site for fluorescence emission.

31. A method according to claim 30, wherein the transporting step includes electrokinetically delivering the intercalating dye.

32. A method according to claim 30, wherein the delivering step comprises hydraulically delivering the intercalating dye.

33. An apparatus for analyzing nucleic acids comprising:

- a microchannel structure formed in a substrate;
- a pattern of reactive, hydrophilic sites disposed in at least a portion of the microchannel structure to produce;
- a plurality of probes coupled to corresponding hydrophilic sites in the microchannel structure; and
- a cover plate affixed to the substrate thus enclosing the probes.

34. An apparatus according to claim 33, further comprising means for electrokinetically transporting a target nucleic acid sample through the microchannel structure to the plurality of probes.

35. An apparatus according to claim 33, further comprising means for hydraulically transporting a target nucleic acid sample through the microchannel structure to the plurality of probes.

36. An apparatus according to claim 33, further comprising means for transporting a target nucleic acid sample to at least one of the probes, thus forming at least one hybridized site, means for delivering an intercalating dye to the hybridized site, and means for probing the hybridized site for fluorescence emission.

37. An apparatus according to claim 33, wherein a surface of the microchannel structure is treated with a hydrophobic substance, thus forming a hydrophobic layer and a non-reactive, hydrophobic surface, at least a portion of which is removed to form a pattern of exposed sites, the exposed sites being treated with a silane coupling agent to produce reactive, hydrophilic sites, exposing oligonucleotide probes to the hydrophilic sites, thereby binding the probes to the hydrophilic sites.

38. An apparatus according to claim 37, wherein the hydrophobic substance is an alkyltrialkoxysilane.

39. An apparatus according to claim 38, wherein the silane coupling agent is selected from the group consisting of 3-aminopropyltriethoxysilane and 3-glycidoxypropyltrimethoxysilane.

40. An apparatus according to claim 8, wherein the oligonucleotide probe is modified with a linking group.
41. An apparatus according to claim 40, wherein the linking group is an aminoalkyl group having the structure: $-(CH_2)_nNH_2$, with the alkyl chain length, n , having a value between 3 and 12, inclusive.
42. An apparatus according to claim 41, wherein the aminoalkyl linking group is attached to the 5' terminus of the oligonucleotide.
43. An apparatus according to claim 41, wherein the aminoalkyl linking group is attached to the 3' terminus of the oligonucleotide.
44. An apparatus according to claim 39, further comprising means for treating 3-glycidoxypropylsilanated sites with an oligonucleotide probe modified with an aminoalkyl group, whereby the aminoalkyl group reacts with the silane epoxide group to bind the said oligonucleotide probe to the silanated site.
45. An apparatus according to claim 39, further comprising means for treating 3-aminopropylsilanated sites with an amine-reactive crosslinker followed by treatment with an oligonucleotide probe modified with an aminoalkyl group, whereby the said oligonucleotide probe is bound to the silanated site.

46. An apparatus according to claim 33, further comprising means for electrokinetically moving target nucleic acids into the channel structure and detecting hybridization between the probes and the target nucleic acids.

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47. An apparatus according to claim 33, further comprising means for hydraulically moving target nucleic acids into the channel structure and detecting hybridization between the probes and the target nucleic acids.

5

48. An apparatus for analyzing nucleic acids comprising:

a microchannel structure formed in a substrate;

a cover plate affixed to the substrate thus enclosing the microchannel structure;

a pattern of reactive sites disposed in at least a portion of the microchannel structure; and

means for applying a different aqueous probe solution to each reactive site to couple a plurality of probes to the microchannel structure.

49. An apparatus according to claim 48, wherein at least a portion of the microchannel structure is treated with a silane linker to produce a non-reactive, hydrophobic surface, and the apparatus further includes a photolabile protective group blocking a functional group of the silane linker, the silane linker being exposed at specific positions in the microchannel to light through the cover plate.

5

50. An apparatus according to claim 49, wherein the silane linker is an aminoalkylsilane.

51. An apparatus according to claim 49, wherein the functional group is the amino function of 3-aminopropylsilane.

52. An apparatus according to claim 48, wherein the probe is an oligonucleotide probe modified with a linking group.

53. An apparatus according to claim 52, wherein the linking group is an aminoalkyl group having the structure: $-(CH_2)_nNH_2$, with the alkyl chain length, n , having a value between 3 and 12, inclusive.

54. An apparatus according to claim 53, wherein the aminoalkyl linking group is attached to the 5' terminus of the oligonucleotide.

55. An apparatus according to claim 53, wherein the aminoalkyl linking group is attached to the 3' terminus of the oligonucleotide.

56. An apparatus according to claim 48, further comprising means for treating 3-glycidoxypropylsilanated sites with an oligonucleotide probe modified with an aminoalkyl group, whereby the aminoalkyl group reacts with the silane epoxide group to bind the said oligonucleotide probe to the silanated site.

57. An apparatus according to claim 48, further comprising means for treating 3-aminopropylsilanated sites with an amine-reactive crosslinker followed by treatment with an oligonucleotide probe modified with an aminoalkyl group, whereby the said oligonucleotide probe is bound to the silanated site.

60. An apparatus according to claim 48, further comprising means for electrokinetically transporting a target nucleic acid sample through the microchannel structure to the plurality of probes.

61. An apparatus according to claim 48, further comprising means for hydraulically transporting a target nucleic acid sample through the microchannel structure to the plurality of probes.

62. An apparatus according to claim 48, further comprising means for transporting a target nucleic acid sample to at least one of the probes, thus forming at least one hybridized site, means for delivering an intercalating dye to the hybridized site, and means for probing the hybridized site for fluorescence emission.

63. An apparatus according to claim 48, further comprising means for transporting a fluorescently-labeled target nucleic acid sample to at least one of the probes, thus forming at least one hybridized site, and means for probing the hybridized site for fluorescence emission.

64. An apparatus according to claim 48, wherein the delivering means includes means for electrokinetically delivering the intercalating dye.

65. An apparatus according to claim 48, wherein the delivering means includes means for hydraulically delivering the intercalating dye.

66. A method for analyzing nucleic acids comprising the steps of:

immobilizing nucleic probes at specific sites within a microchannel structure; and

moving target nucleic acids into proximity to the probes in order to allow hybridization and fluorescence detection of specific target sequences.

67. A method according to claim 66, wherein the moving step includes an electrokinetic force within the microchannel structure.

68. A method according to claim 66, wherein the moving step includes applying a hydraulic force to the microchannel structure.

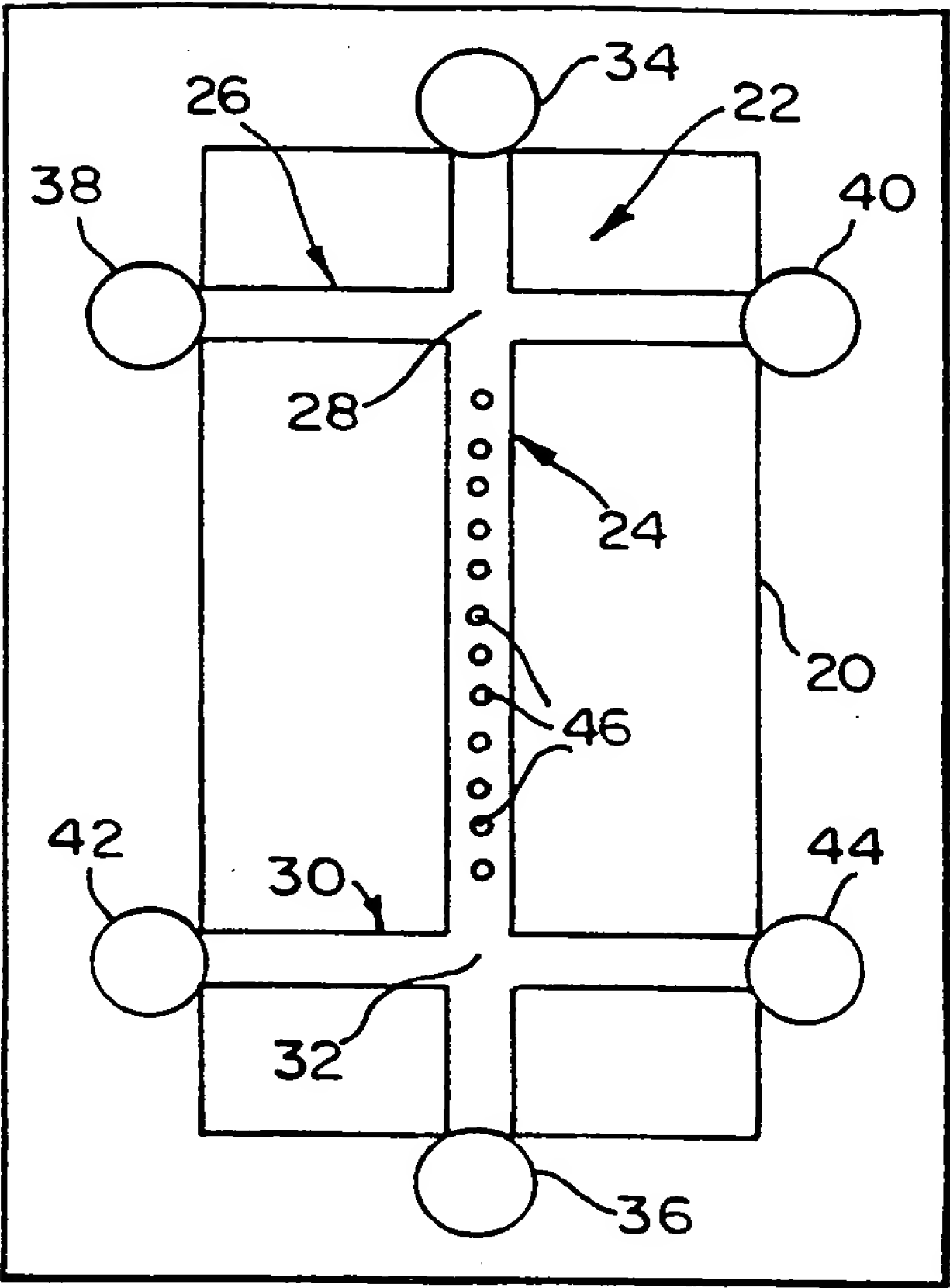


FIG. 1

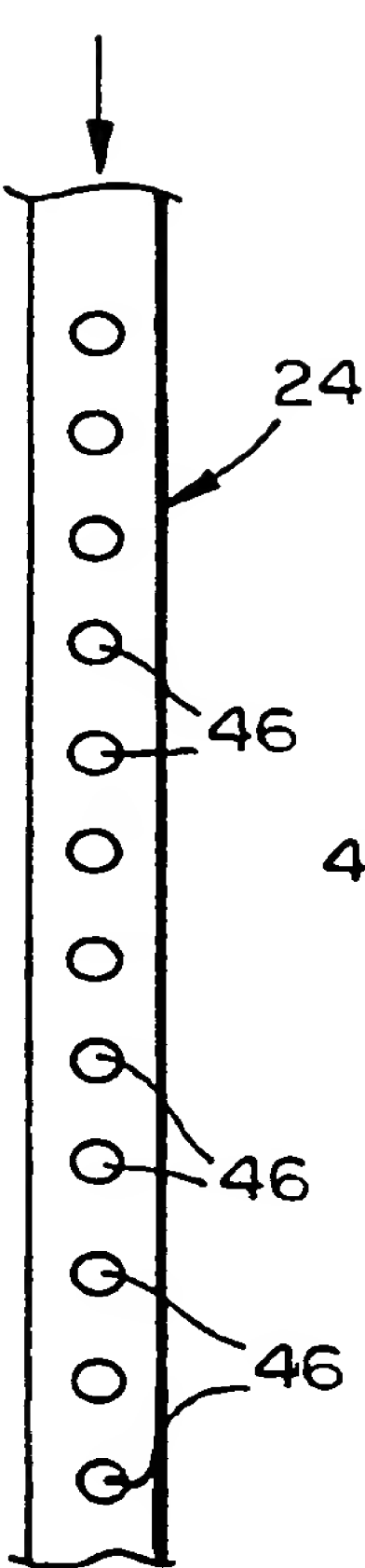


FIG. 2

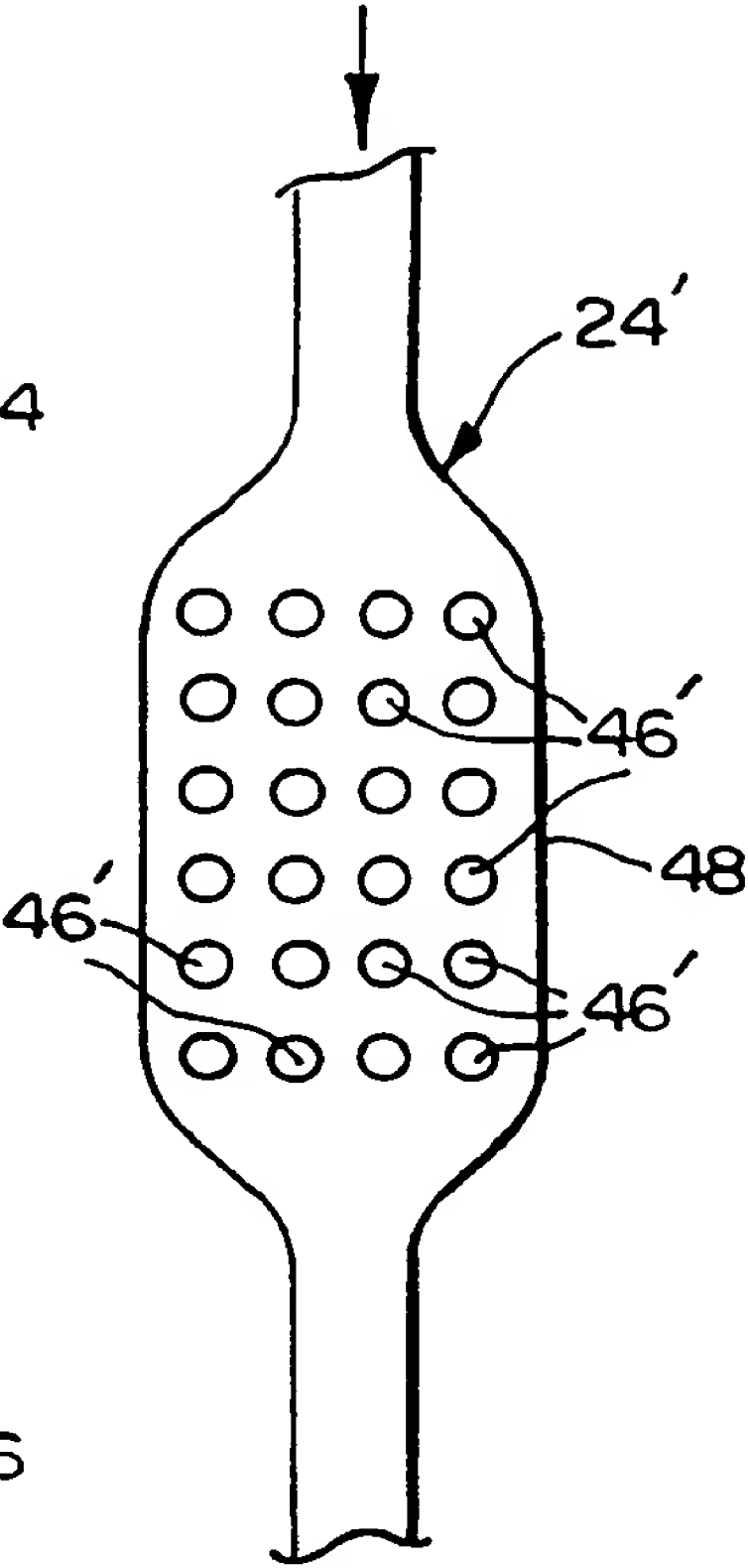


FIG. 3

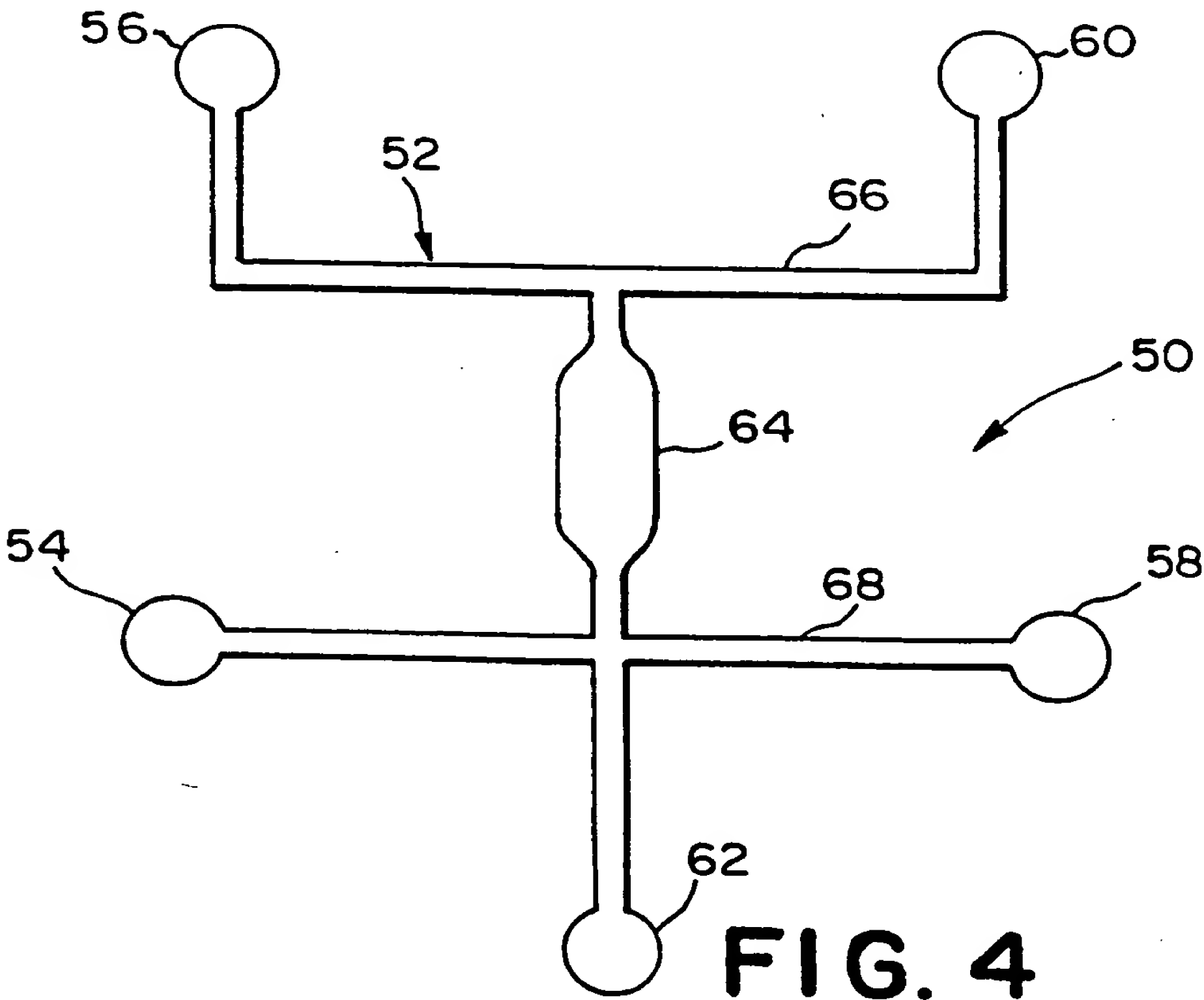


FIG. 4

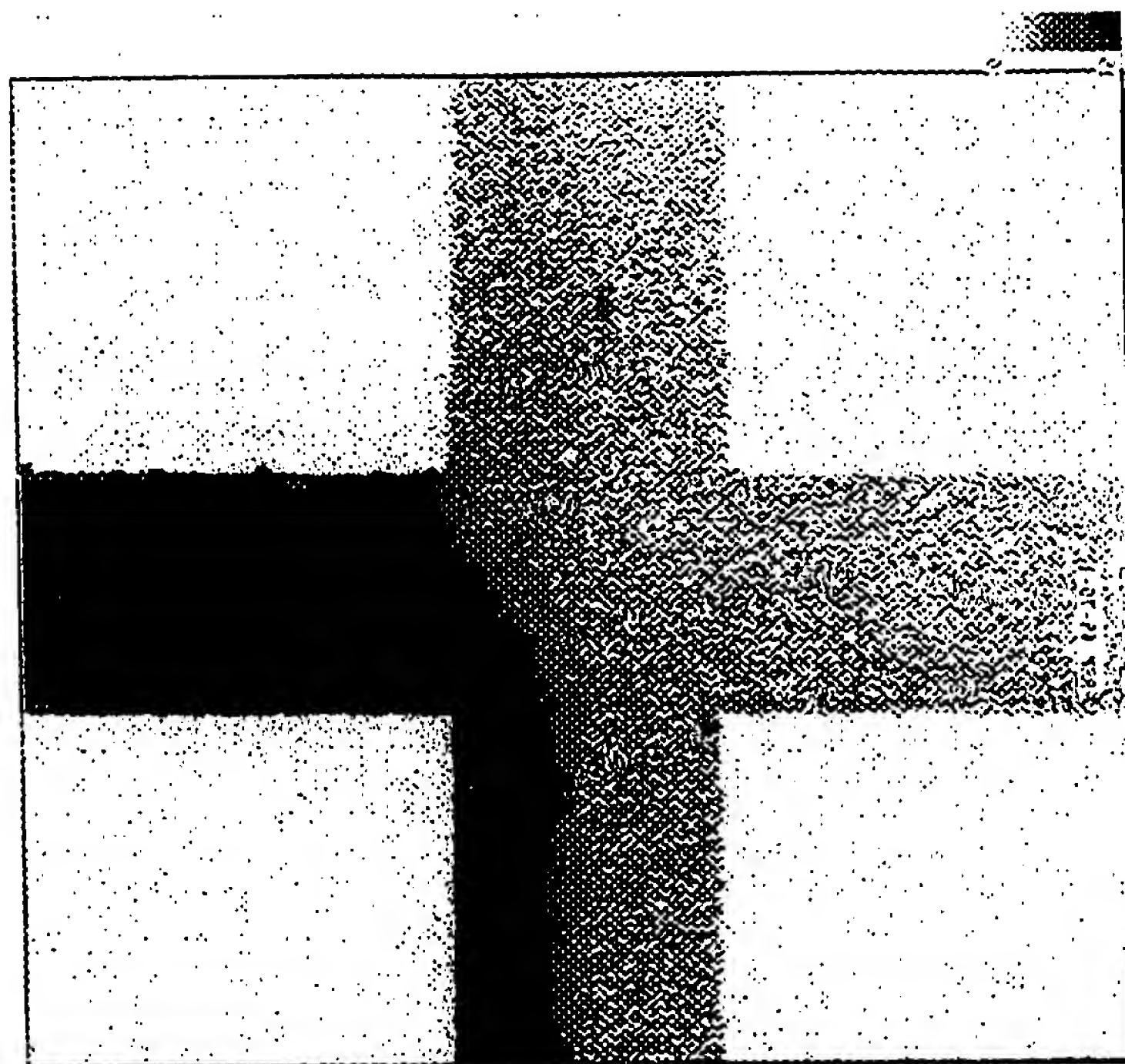


FIG. 6

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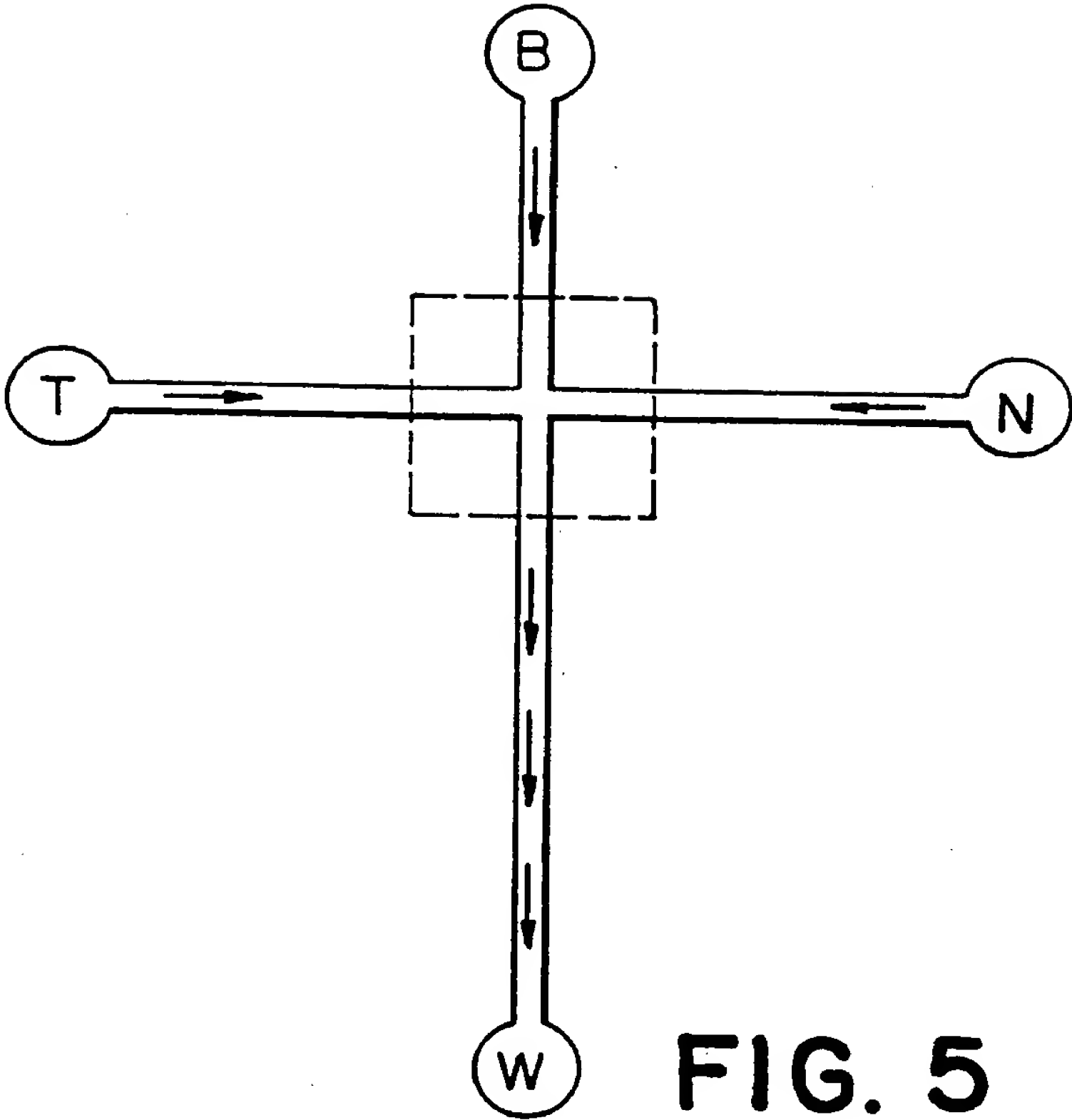


FIG. 5

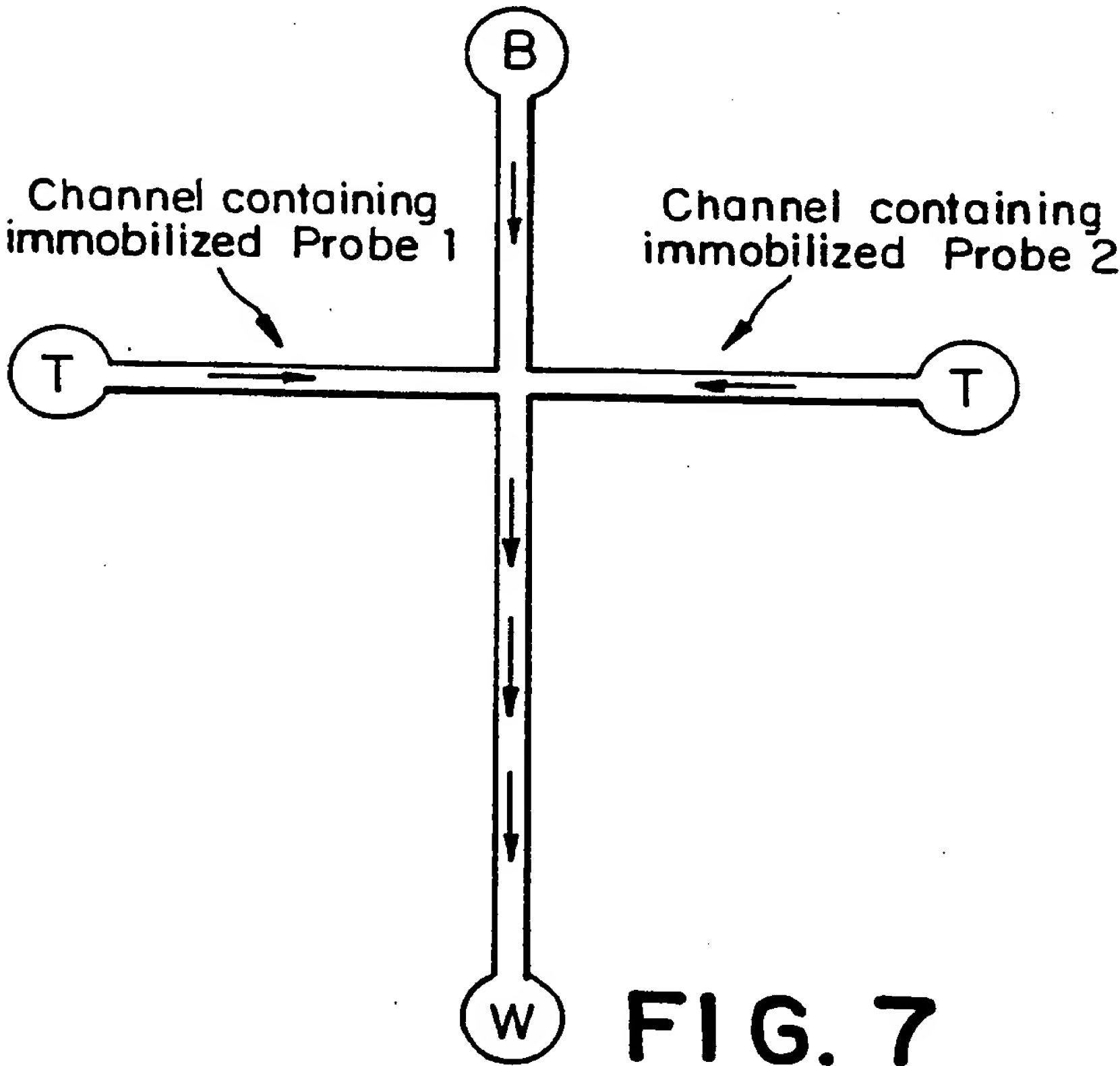
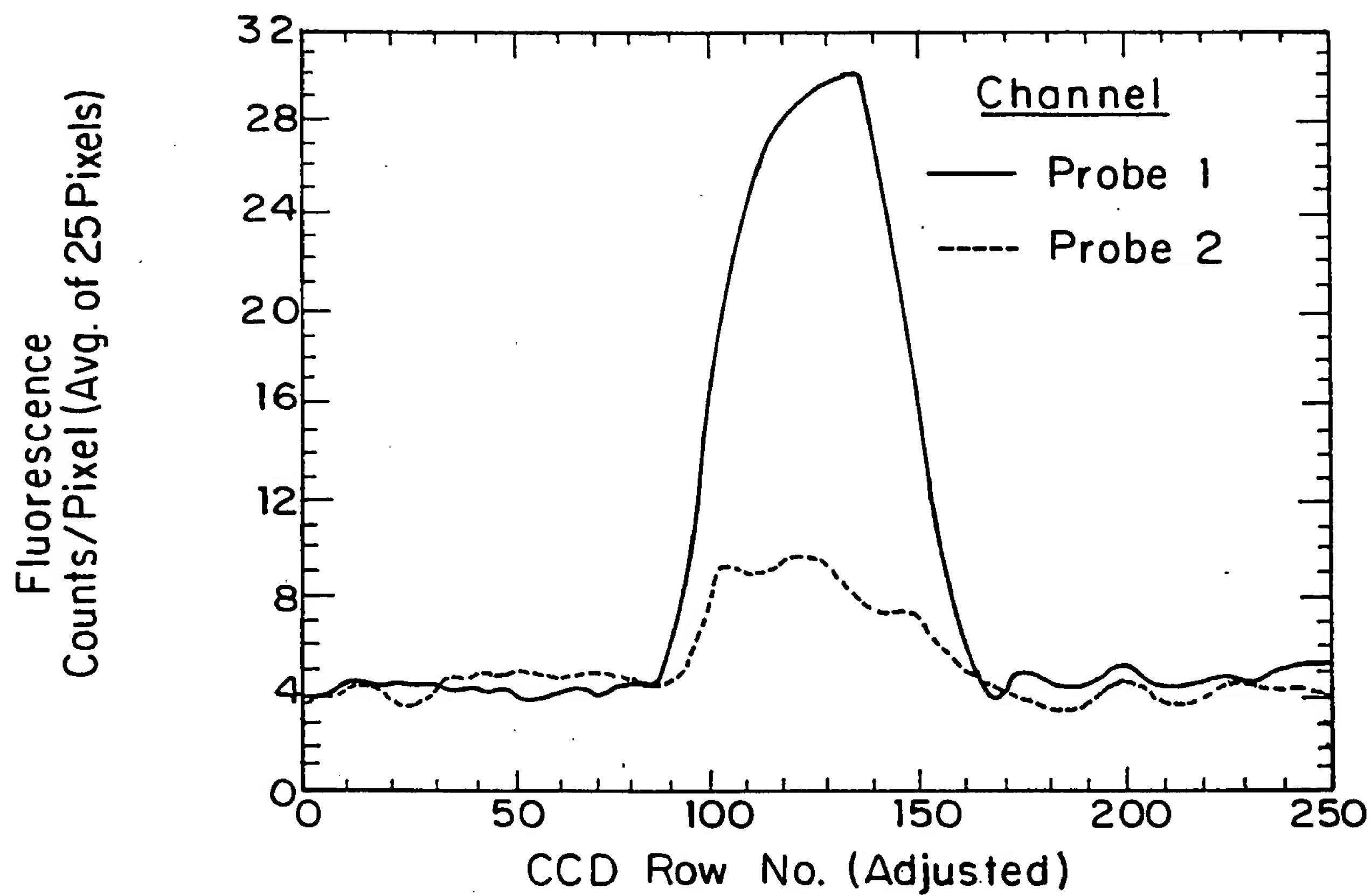


FIG. 7

**FIG. 8**

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/08461

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C12Q 1/68; G01N 33/551, 33/558, 33/569

US CL :435/6, 283.1, 287.1, 288.2, 288.7; 422/58, 68.1, 82.08; 436/94

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 283.1, 287.1, 288.2, 288.7; 422/58, 68.1, 82.08; 436/94

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, MEDLINE, BIOSIS, CA, DERWENT

search terms: microchannel, microfluidic, microchip, hybridize, probe

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|-----------|---|-----------------------|
| Y | US 5,304,487 A (WILDING et al.) 19 April 1994, cols. 5-9. | 1-68 |
| A | US 5,498,392 A (WILDING et al.) 12 March 1996. | 1-68 |
| A | WILDING et al. Manipulation and flow of biological fluids in straight channels micromachined in silicon. Clin. Chem. 1994, Vol. 40, No. 1, pages 43-47. | 1-68 |



Further documents are listed in the continuation of Box C.



See patent family annex.

| | |
|---|--|
| * Special categories of cited documents: | *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention |
| *A* document defining the general state of the art which is not considered to be of particular relevance | *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone |
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| *O* document referring to an oral disclosure, use, exhibition or other means | |
| *P* document published prior to the international filing date but later than the priority date claimed | |

Date of the actual completion of the international search

16 JULY 1998

Date of mailing of the international search report

01 SEP 1998

Name and mailing address of the ISA/US
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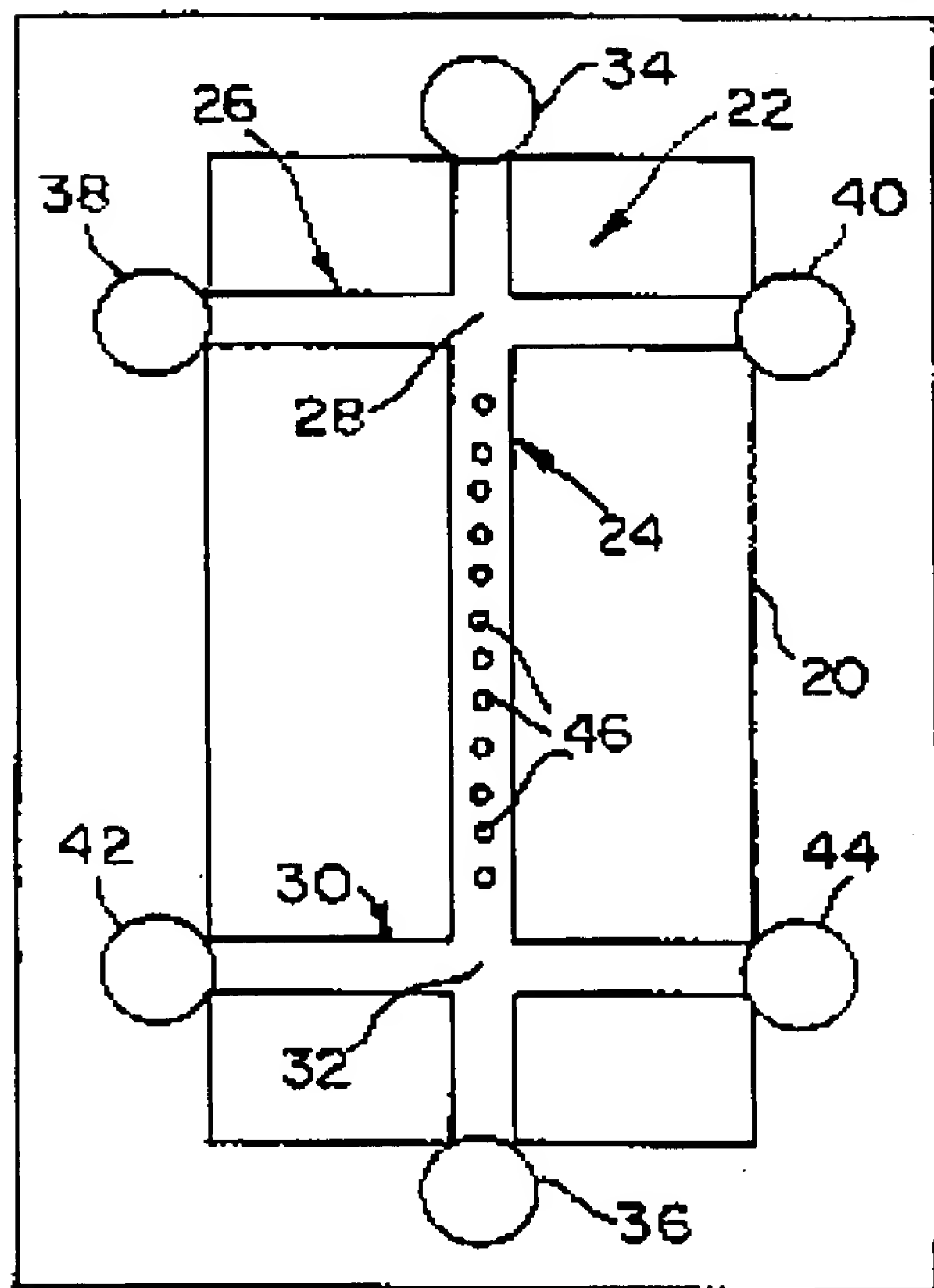


FIG. 1

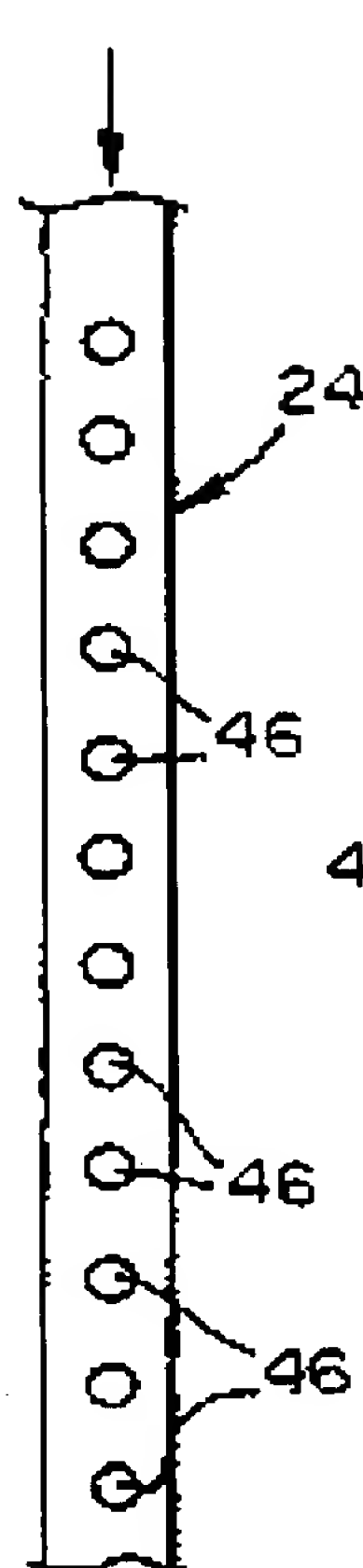


FIG. 2

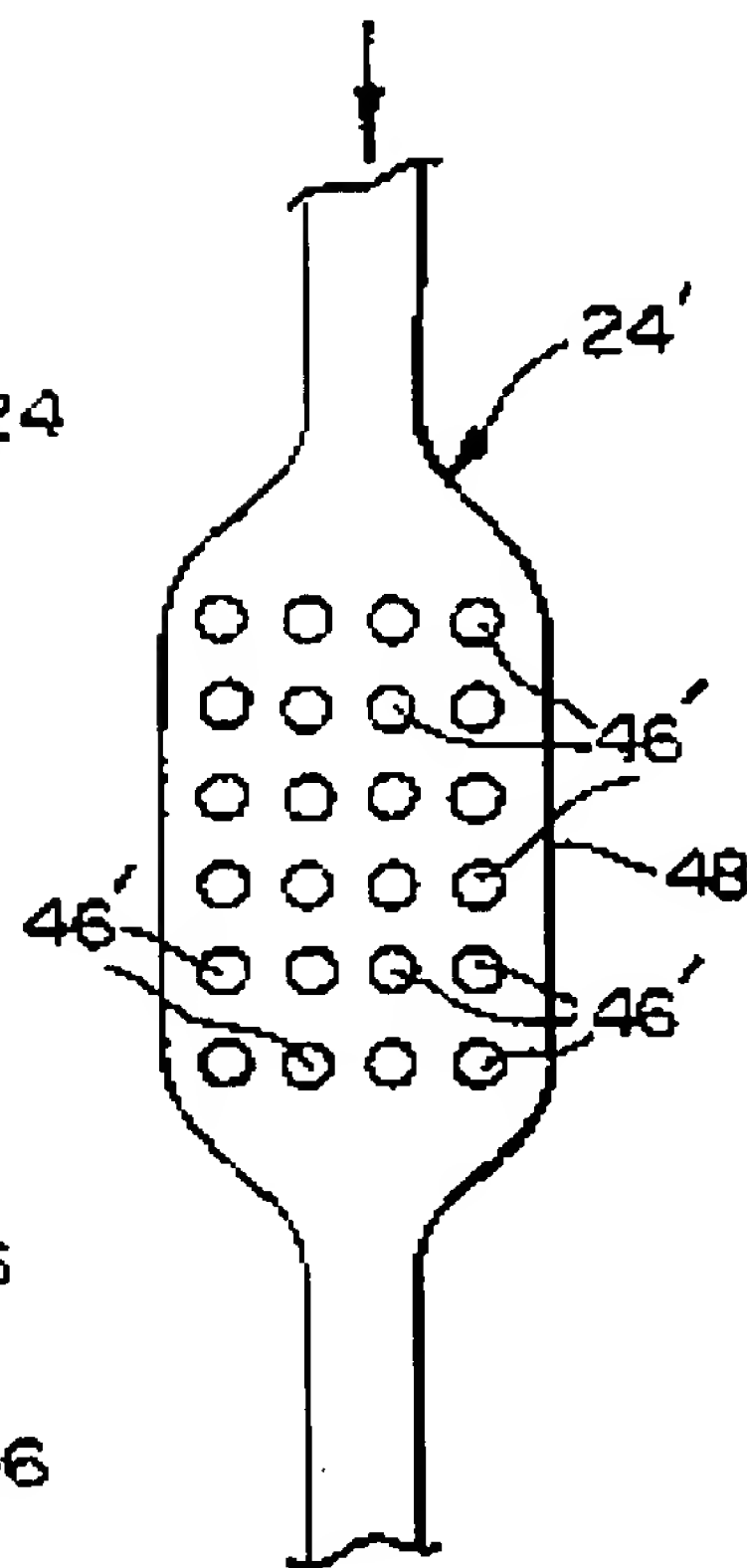


FIG. 3

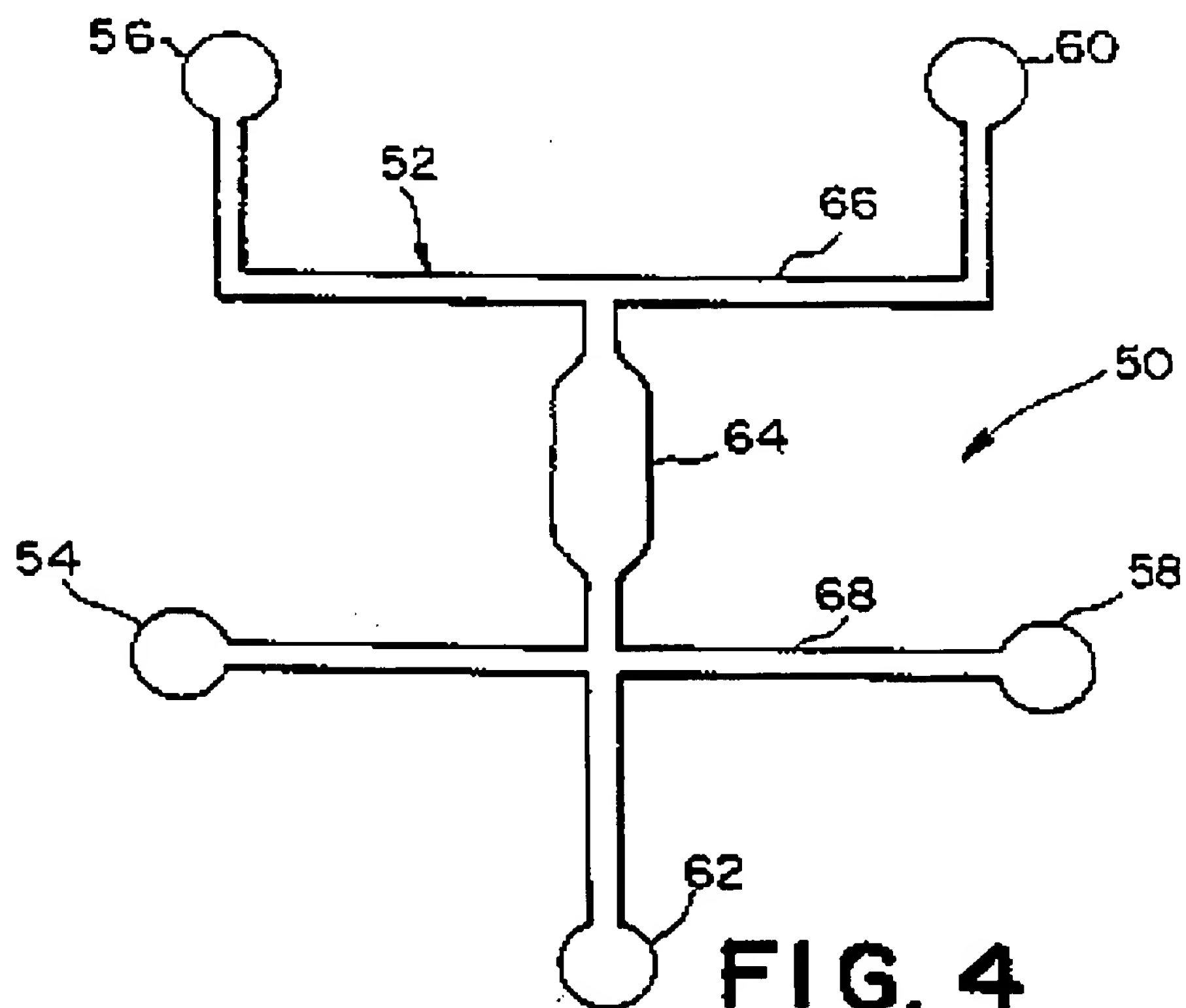


FIG. 4

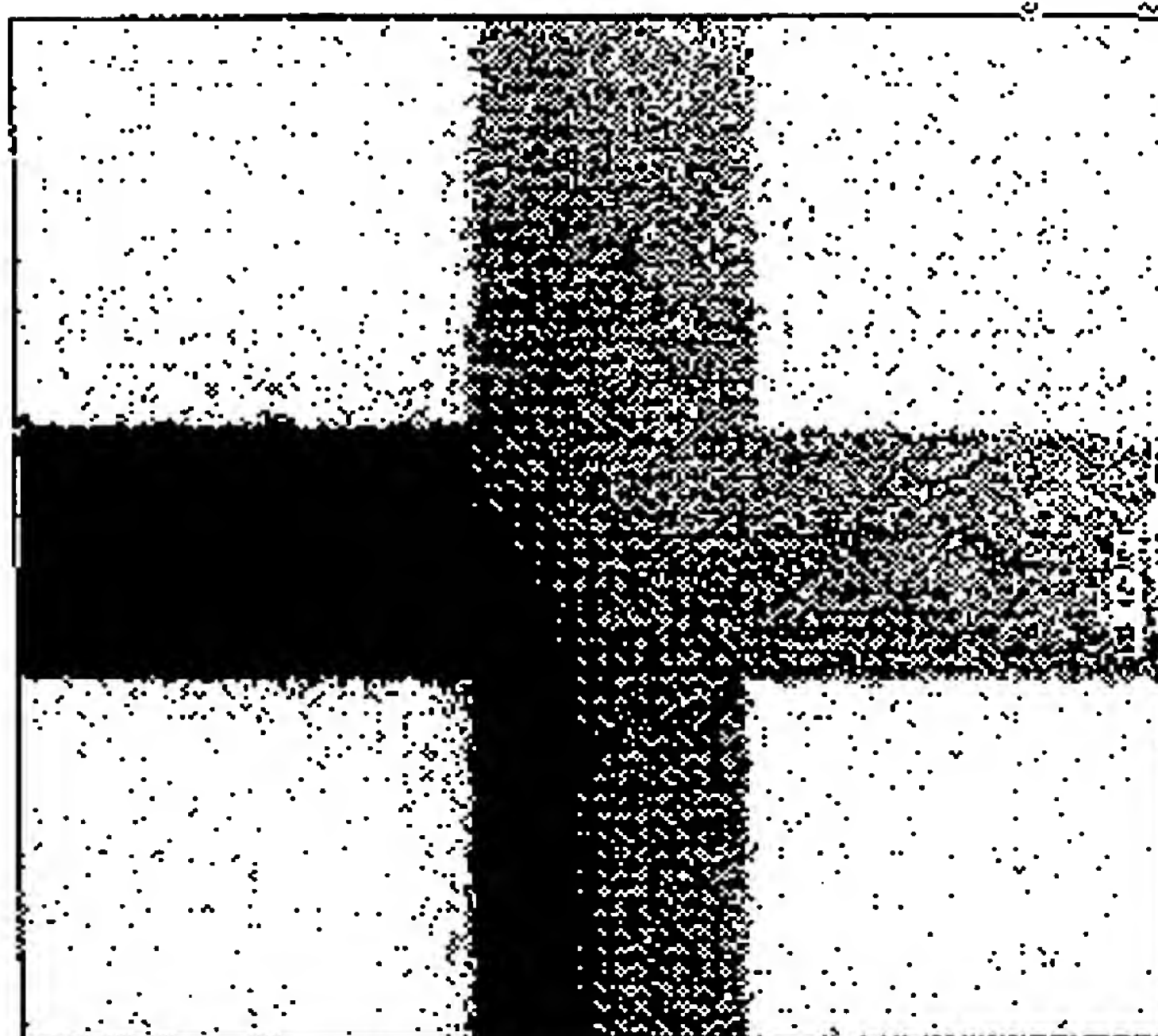


FIG. 6

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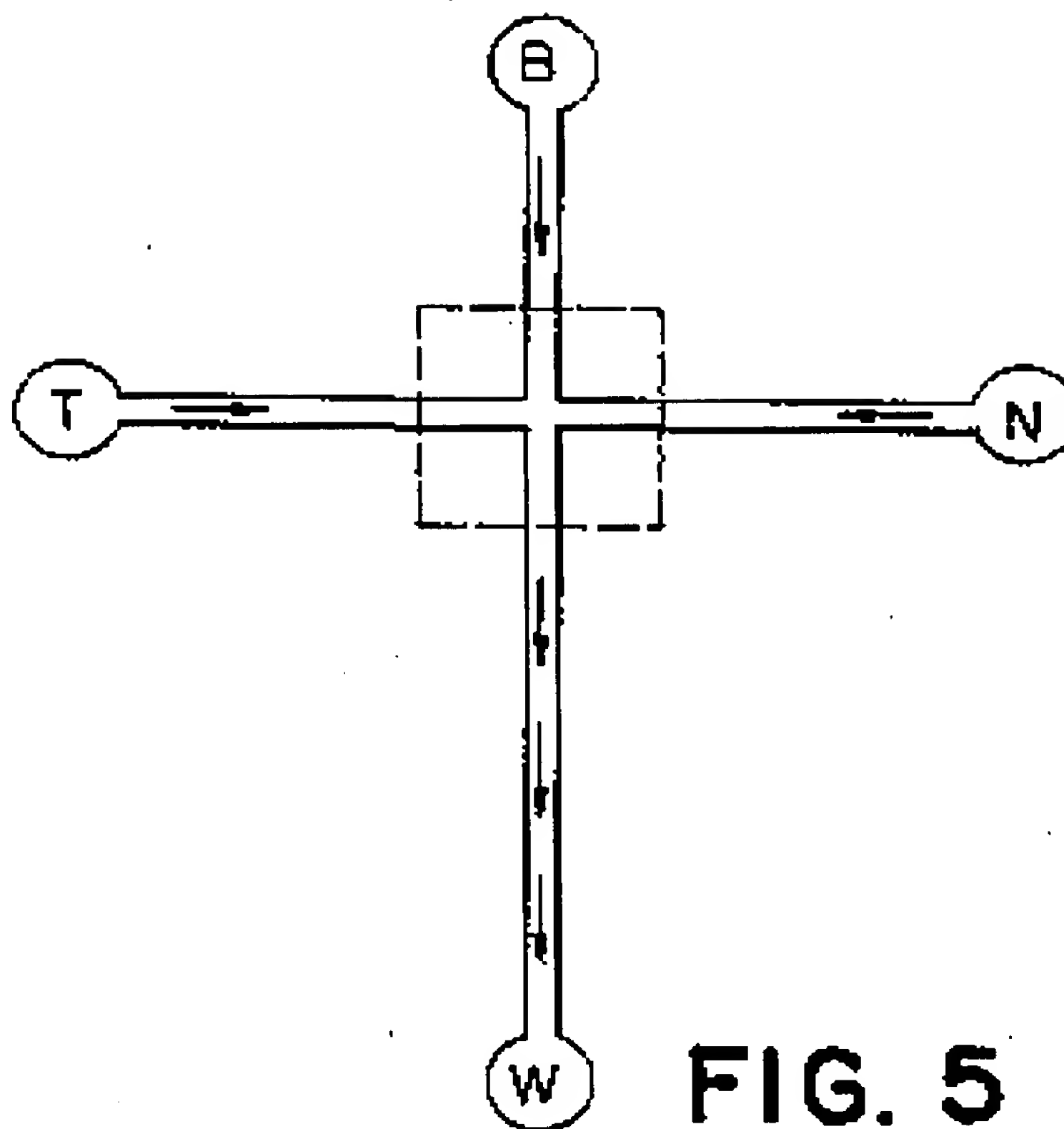


FIG. 5

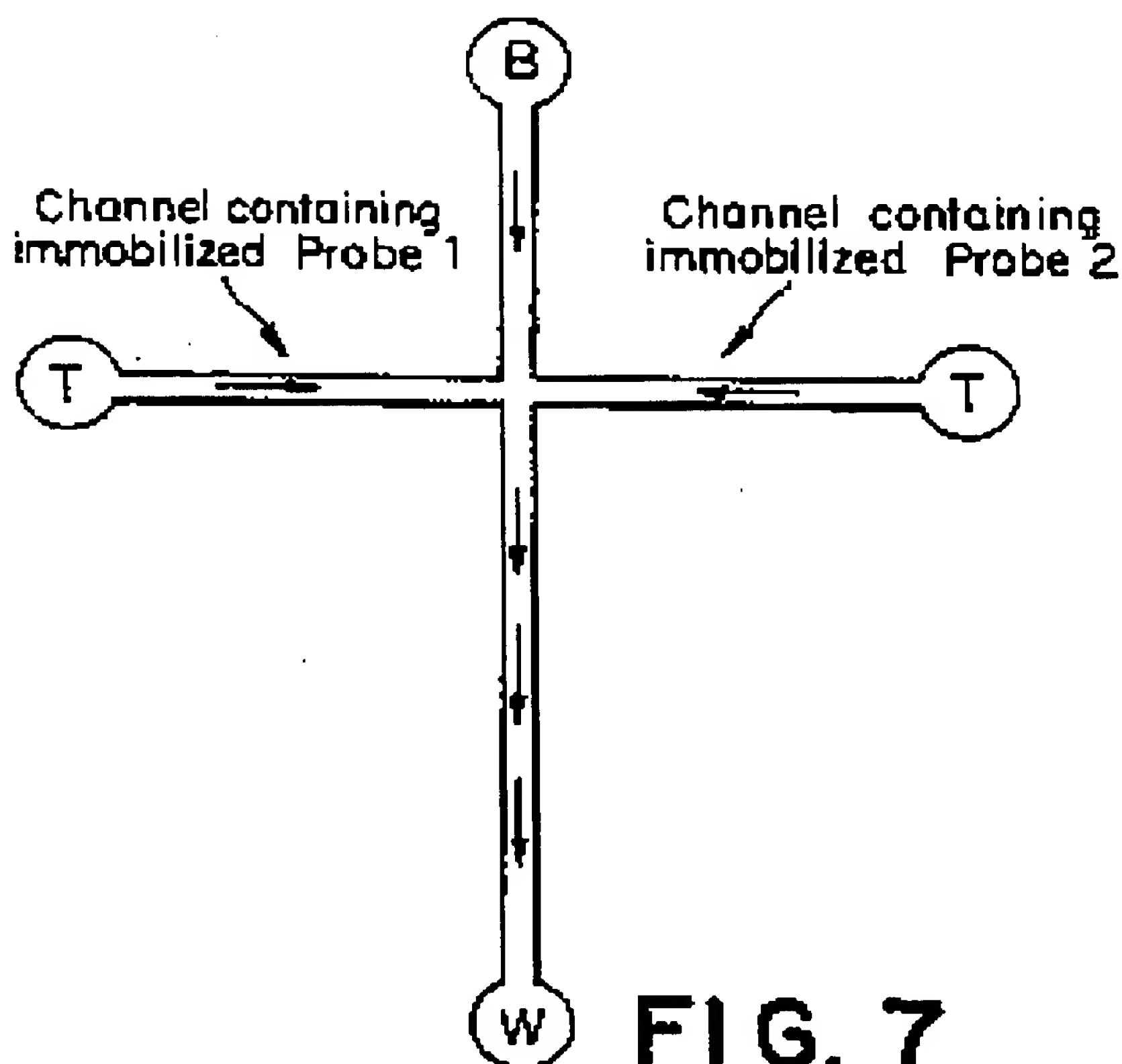
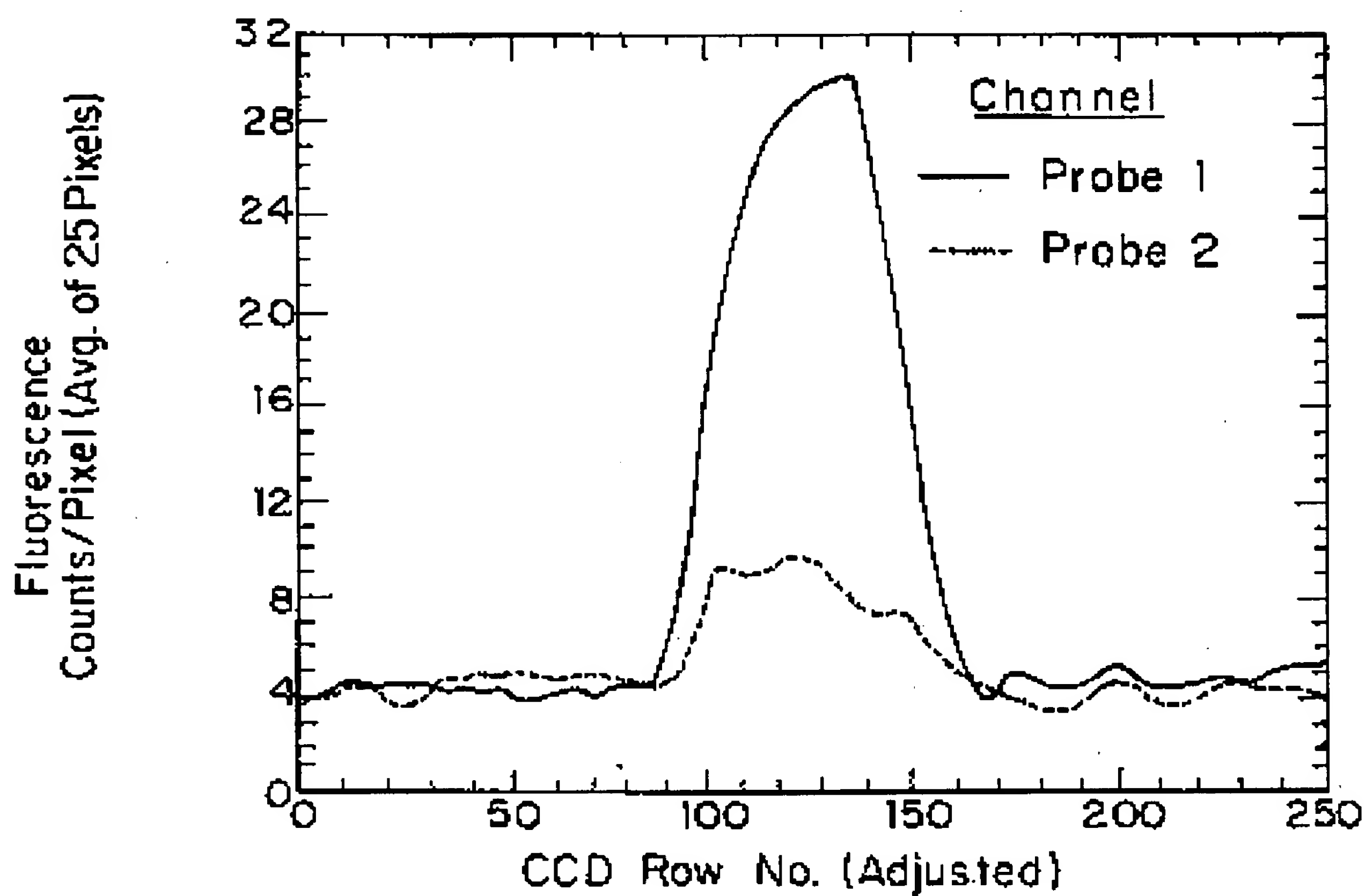


FIG. 7

**FIG. 8**